Supplementary Method 1

Immunohistochemistry

Inflammation

The iliac artery was removed and placed into formalin. Segments of the artery were fixed in 10% (v/v) formalin solution and embedded in paraffin. Cross sections (4 μm) were cut at the iliac and placed at 24°C for 12 hours to allow tissue to adhere to the slide. Sections were immunostained with an antibody against the inflammatory markers, receptor for advanced glycation end products (RAGE; Dilution 1:1000, LSBio, Seattle, WA, USA), HMGB1 (Dilution 1:500, NOVUS, Littleton, CO, USA), or TNF-α (Dilution 1:200, ABCAM, Cambridge, MA, USA) with exposure at 4°C overnight. The primary antibody was labeled with a biotinylated link antibody directed against the mouse antigen using a peroxidase-base kit (LSAB, DAKO, Santa Clara, CA, USA), and visualized by diaminobenzidine (DAB, DAKO) substrate with enhancer. The sections were subsequently counterstained with hematoxylin (DAKO). Computer program-assisted (LAS V4.2) color image analysis segmentation with background correction was used to quantify the immunohistochemistry stains of RAGE, HMGB1, and TNF-α positive-inflammation. Arteries were selected from each treatment group and compared with an adjacent control vessel. RAGE, HMGB1, and TNF-α staining was then quantified within the media, intima, and plaque. The percentage of positive staining was expressed as a function of total plaque area.

Macrophage

Sections were immunostained with an antibody against the smooth muscle cell marker RAM11 (Dilution 1:200, DAKO) with exposure at 4°C overnight. The primary antibody was labeled with a biotinylated link antibody directed against the mouse antigen using a peroxidase-base kit (LSAB, DAKO), and visualized by diaminobenzidine (DAB, DAKO) substrate with enhancer. The sections were subsequently counterstained with hematoxylin (DAKO). Computer program-assisted [Leica Application Suite (LAS) 4.2 (Leica, Wetzlar, Germany)] color image analysis segmentation with background correction was used to quantify the immunohistochemistry stains of RAM11 positive-macrophages. Arteries were selected from each treatment group and compared with an adjacent control vessel. The percentage of positive staining was expressed as a function of total vessel area.